



Figure 1A

SEQ ID NO:1

/translation="MGSVLSTDSGKSAPASATARALERRRDPELPVTSFDCAVCLEVEL
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DRSLLEYVNHSNTT"

Figure 1B

SEQ ID NO:2

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Figure 1C

SEQ ID NO:3

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Figure 1D

SEQ ID NO:4

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Figure 1E

SEQ ID NO:5

TRAC1 genomic region:

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Figure 1F

SEQ ID NO:6

Mouse TRAC1 cDNA sequence:

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SEQ ID NO:7

Mouse TRAC1 protein (3rd frame)

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ELDEDCLLDHCI IHRSERRPVFCPLCHSRPDESPSTFNGSLIRHLQVSH
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FIGURE 2A

FLJ20456 Hit Inhibited anti-TCR Induced CD69 Expression in Jurkat Cells



Original clone

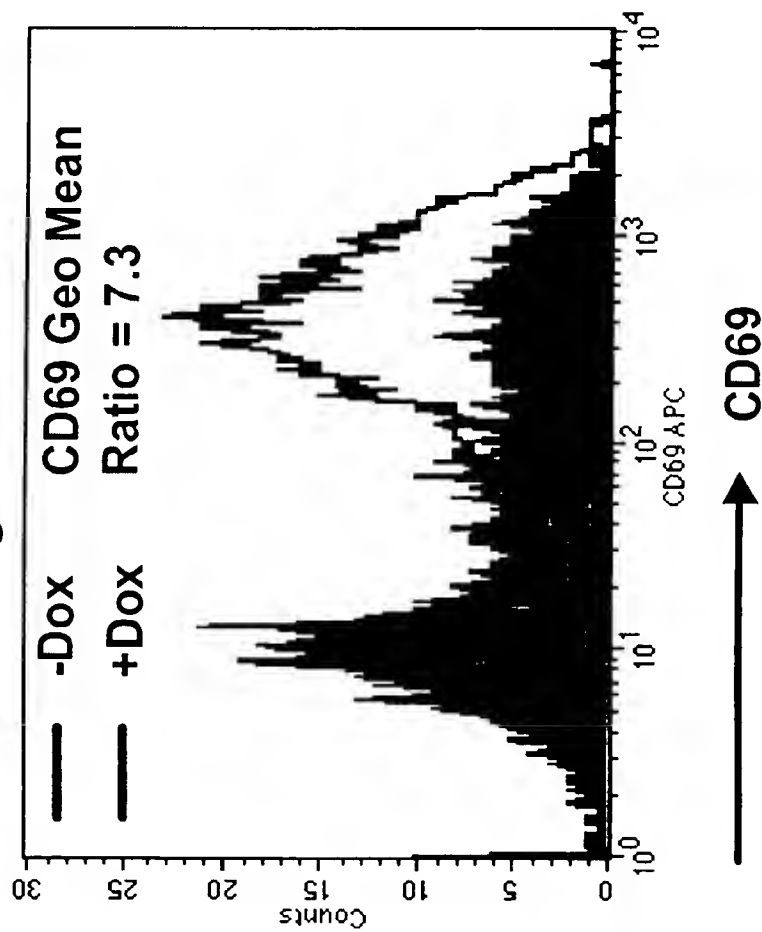


FIGURE 2B
FLJ20456 Hit Inhibited anti-TCR Induced
CD69 Expression in Jurkat Cells
Phenotype Transfer

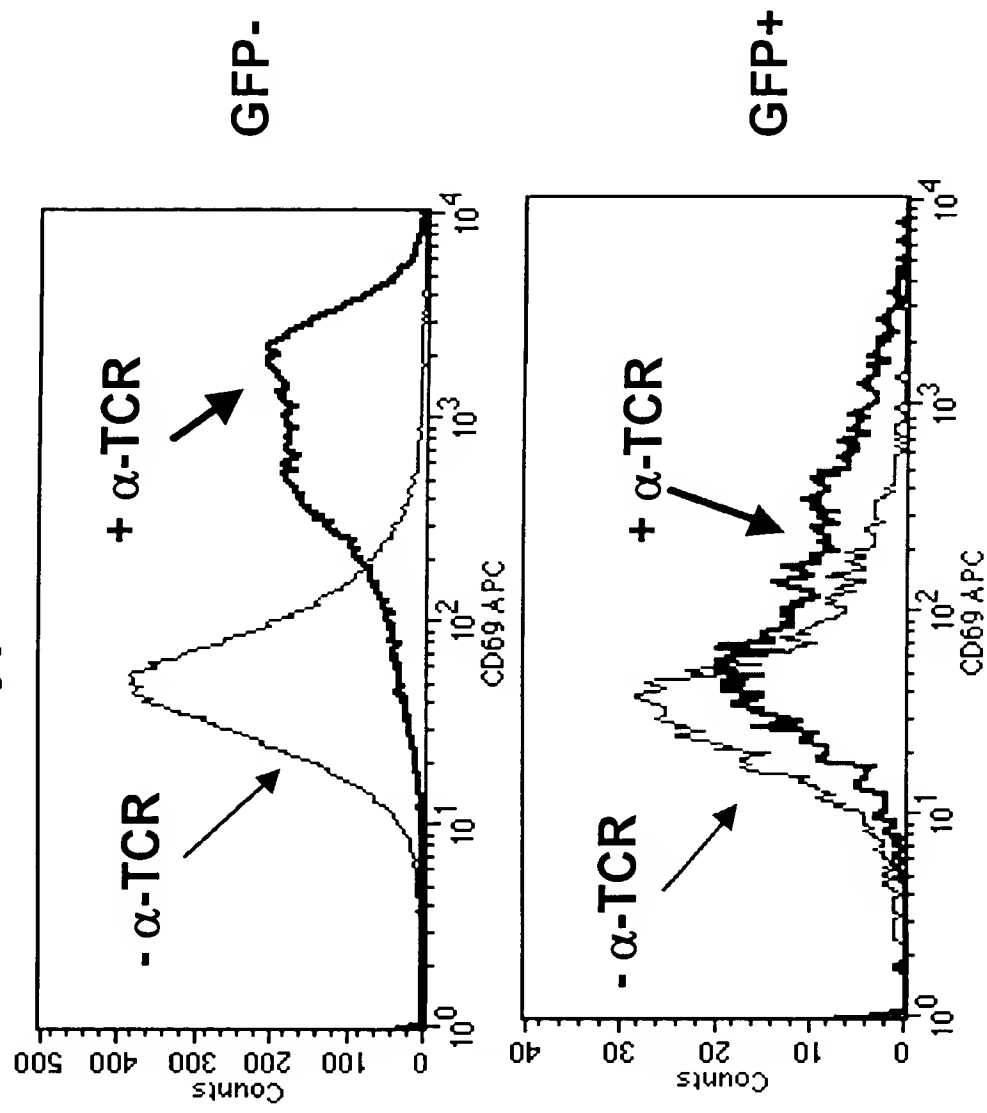


Figure 3A

Full Length FLJ20456 Does Not Inhibit CD69 Upregulation in Jurkat Cells



- Pfu PCR product amplified from a capped human brain cDNA library.
- One N to S polymorphism with FLJ20456 NM_017831.1 at amino acid 186, present in EST database.

JurkatN 32H

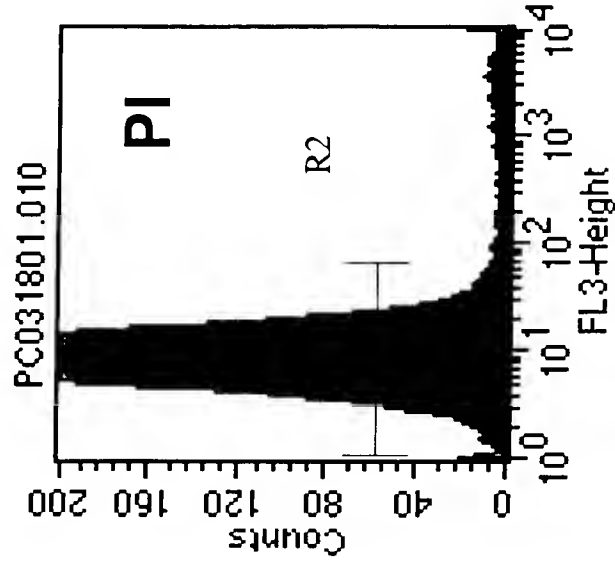
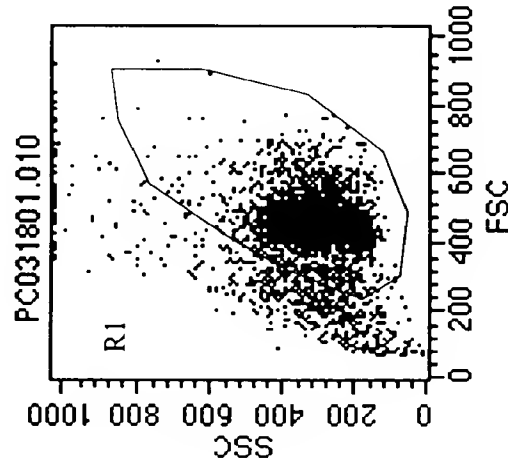
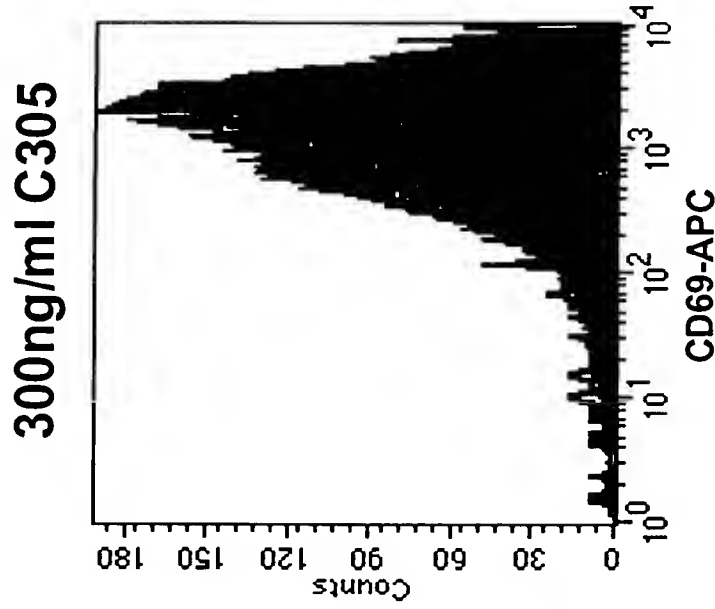
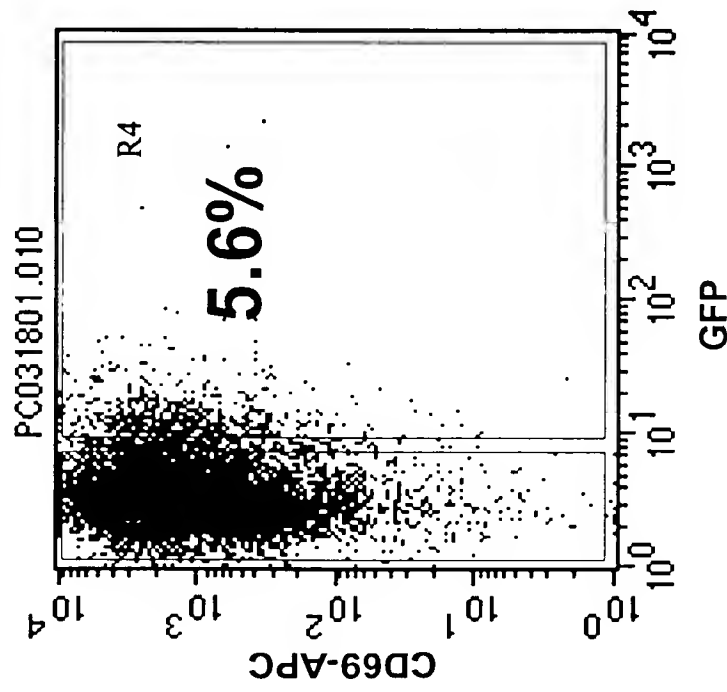


Figure 3B

Full Length FLJ20456 Does Not Inhibit CD69 Upregulation in Jurkat Cells



GFP- 1070.5

GFP+ 1219.9

Ratio = 0.88

Figure 4A

FLJ20456 Hit Specifically Inhibited T Cell Activation but not B Cell Activation

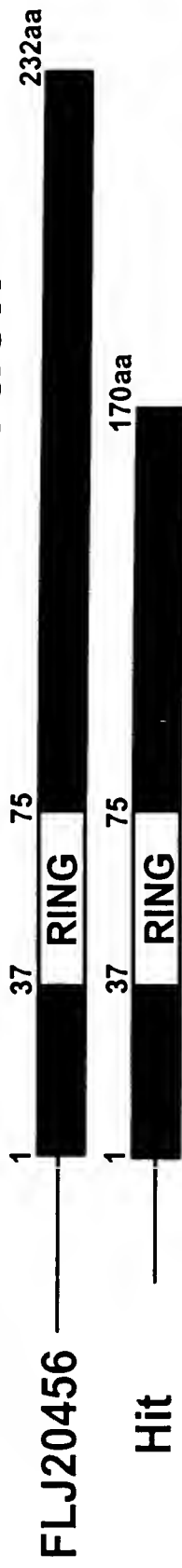


Figure 4B

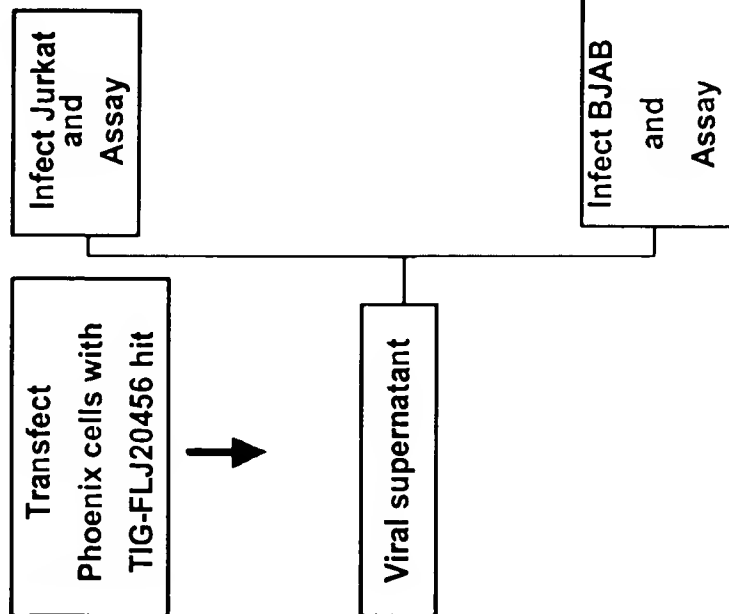
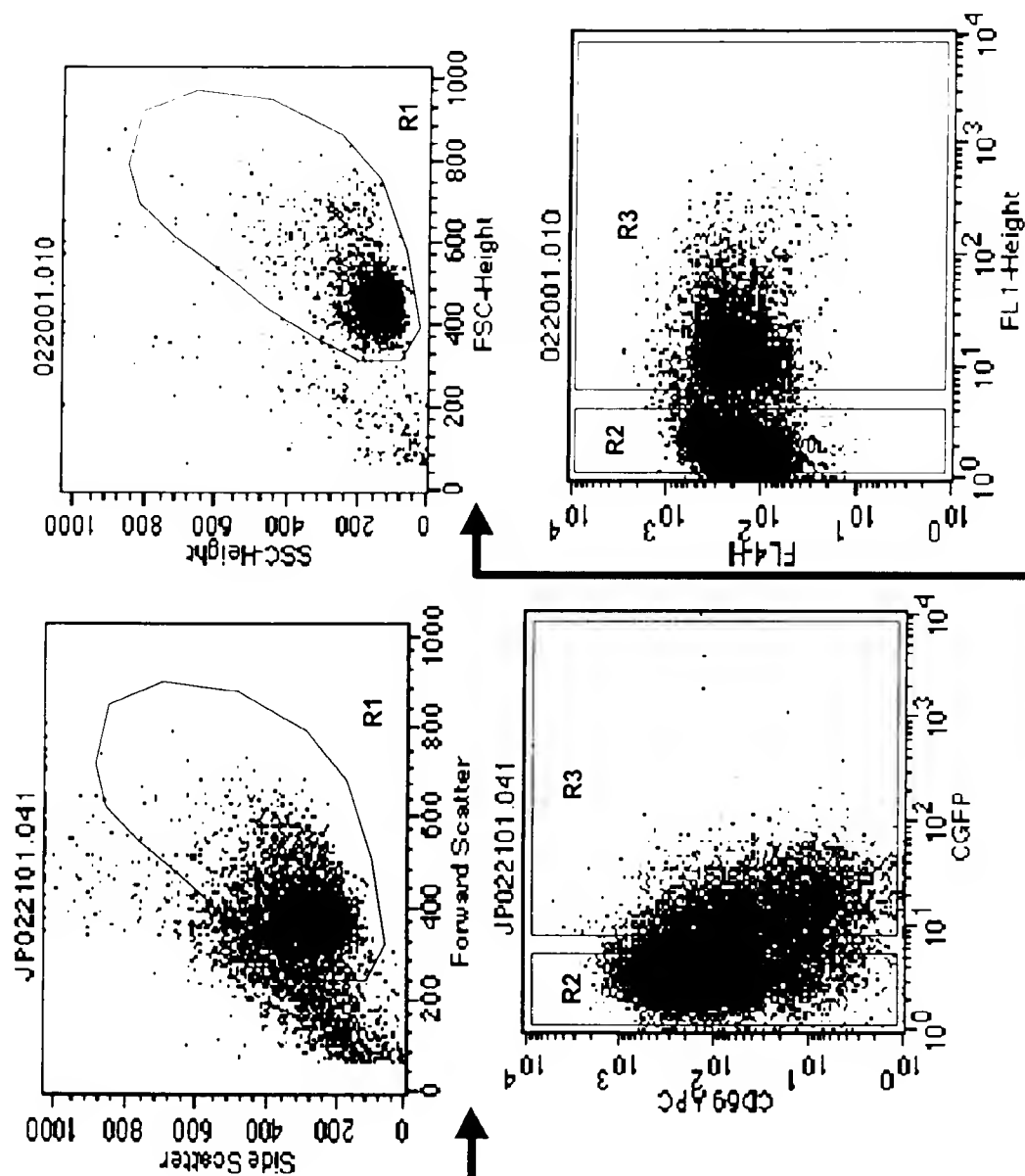
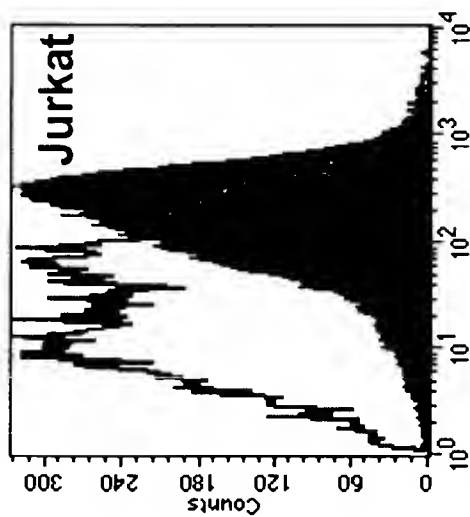


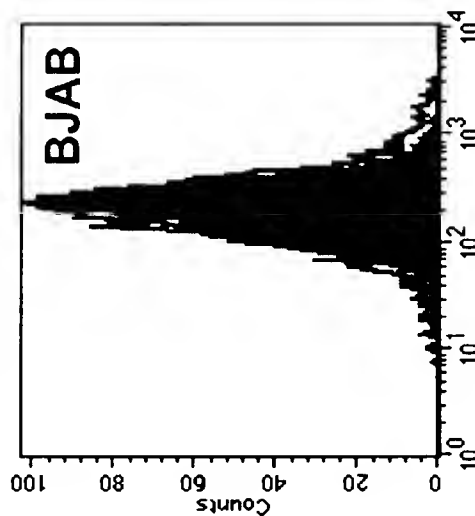
Figure 4C



CD69-APC

Stimulation:
300ng/ml C305

GFP-	141.2
GFP+	28.8
Ratio =	4.9



CD69-APC

Stimulation:
300ng/ml α -IgM

GFP-	183.1
GFP+	183.3
Ratio =	1.0

Figure 5

FLJ20456 Is Strongly Expressed in Lymphoid and Hematopoietic Organs

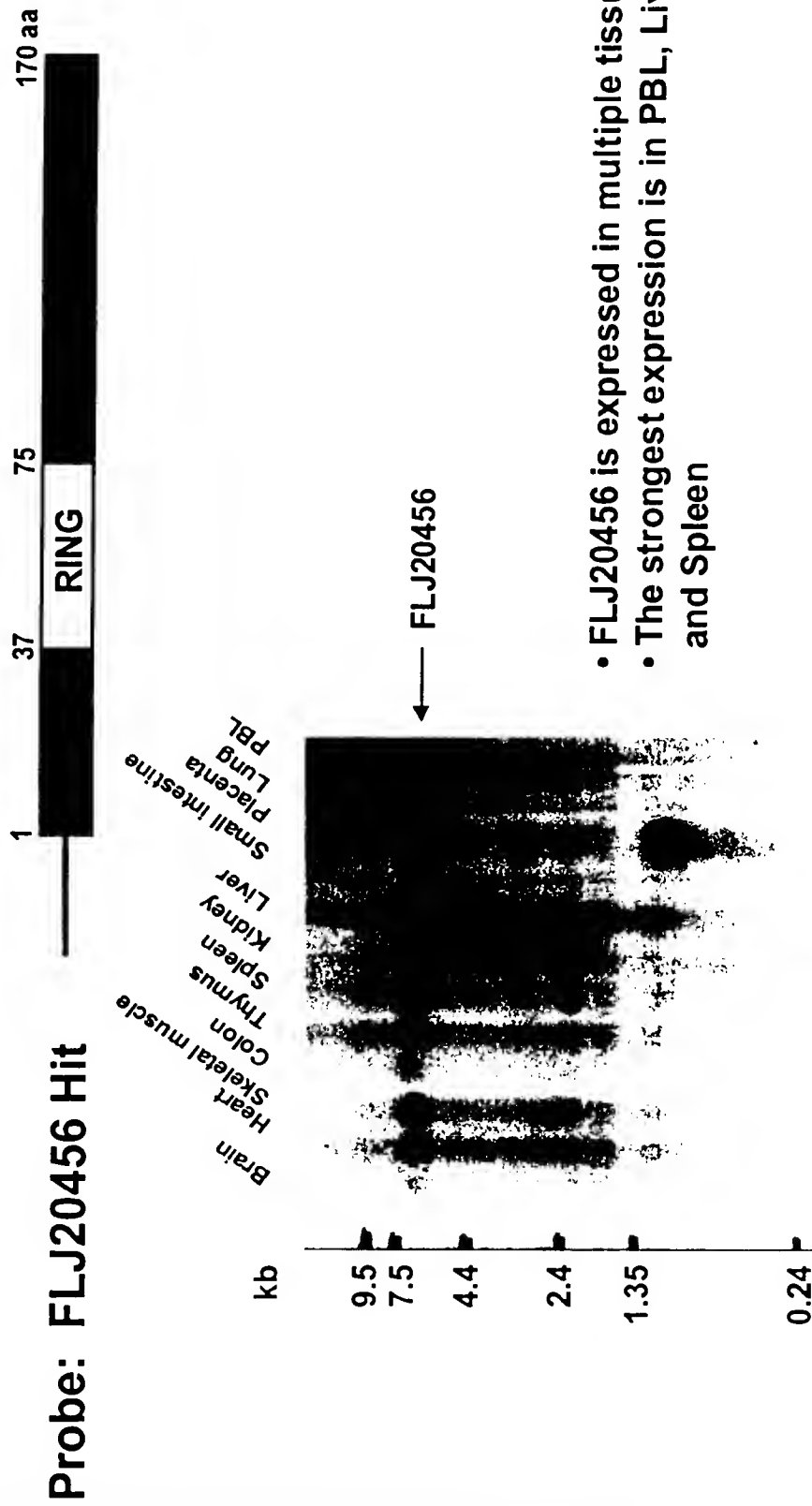
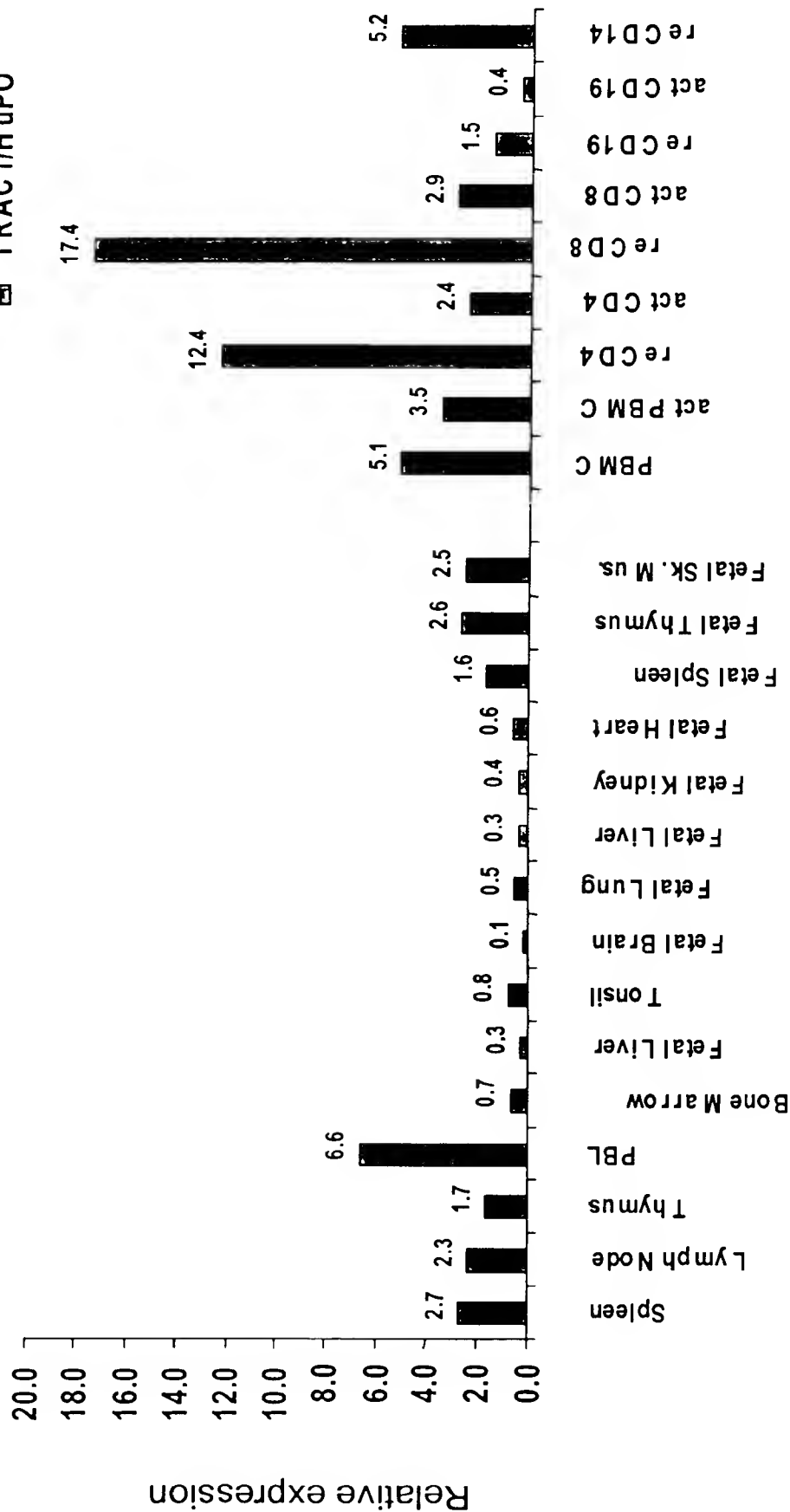


Figure 6

TRAC1 is Predominantly Expressed in Resting T lymphocytes

Primer/Probe SetA

TRAC1/HuPO



FL20456 Sequence is Most Similar to Two Sequences: Znf313 and STRIN

Consensus #1F.C.VC.EV...PV....C.HVFC..C.....

Ring Domain

FLJ20456.pep MSSVLSTUSGKSAPASATARAATERRRRDPVTSFDCAVCLVLTQPVRTRCGHVFCRSIAATSLKN 67
 znf313.pep -----MAAQQRDCGGAQCLAGPAAEADPLGRFTCEVCLVEYKPVQV-PCGHVFCSAQLQECLEKP 59
 STRIN.pep -----MAEDLSAATSYTEDDFVCPVCEVLTQPVRTTACQCHVFCRKCFLTAMRE 49

Consensus #1C..CR.....A.....C..C.....R.H...C.KY...Y.....

FLJ20456.pep NKWTCEYCRAYLPSE----GVPAATDVAKRNMKSEYKNCAECDTLVCLSEMAFAIRTOOKYIDKYGPLQE 131
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 STRIN.pep SGAHCEPLCPGNVTRRERACPEPALDLENIMRKFSGSCROCAKQIKFYRMHHYKSKKYQDEYGVSSI 117

Consensus #1CP.C.....L.HC....H.....

FLJ20456.pep LEETAAR-----CVCFFQRELVE-DSLLDHGITHIRRSERR 166
 znf313.pep KATIKDASLQPRNVPNRY-----TFPCPYCEPEKNFDQEGLEVHCKLFTSTDTK 169
 STRIN.pep VPNFQISQDSVGNNSNRSETSTSDNTETYQENTSSSGHPTFKCPLCQESNFTQRQLLDHCNSNHLFQIV 185

Consensus #1 .V.CP.C...P...P.....N...H.....Y..F.....EE.....S.....--

FLJ20456.pep PMFCPLLCRLIPDENPSSFSGNLIRHLQVSHLTLYDDFLDFNIIEEALIRRVLDRLSLLLEYVNSHTT. 233
 znf313.pep SMVCPICASMPWGDENYRSANERHILQRFHFSMDTFVDYDVEEDMMNQVLQRSIIDQ. 229
 STRIN.pep PMTCTICVSLPWGDPSQITRNEVSHLNQRFQFDYGEEMNLQIDDEETQYQTAVEESFQVNI. 246

* All three sequences are human
 * Murine sequences are not shown

	1	2	3	
1		26.6	22.3	1
2	130.4		27.9	2
3	140.9	134.7		3
	1	2	3	
				FLJ20456.pep
				znf313.pep
				STRIN.pep

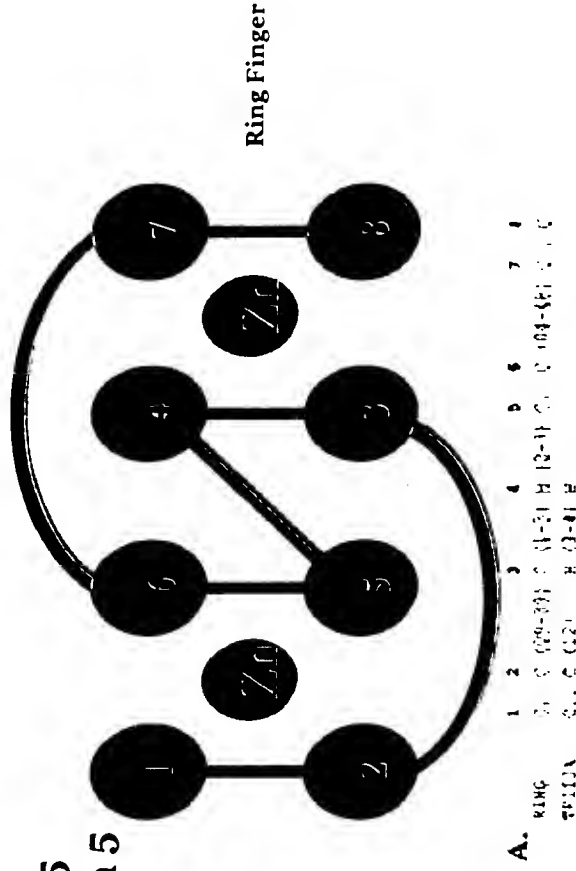
FIG. 7.

RING finger vs. Zinc finger proteins

Figure 9

Ring-HC: C_3HC_4 = Cys in position 5
 Ring H2: $C_3H_2C_3$ = His in position 5

- Ring finger domains have a conserved pattern of Cys and His residues that coordinate two zinc atoms to form a cross-brace structure



- Ring fingers are structurally distinct from zinc fingers

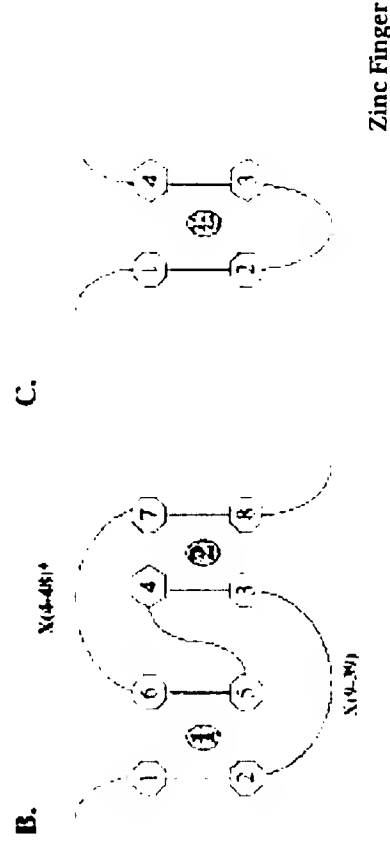


Figure 10A

Ubiquitin Pathway Components

- E1: ubiquitin-activating enzyme, with a major isoform that may work broadly
- E2: ubiquitin-conjugating enzyme, a class of ~14 enzymes, interacts with E3
- E3: ubiquitin ligases, a broad and growing group of activities that promote addition of ubiquitin to specific proteins
- Proteasome-a 26S complex containing a 19S lid and base that mediates ATP- and ubiquitin-chain-dependent binding of substrates and a 20S catalytic core with three known proteolytic activities.

Enzymology of Ubiquitination

Figure 10B

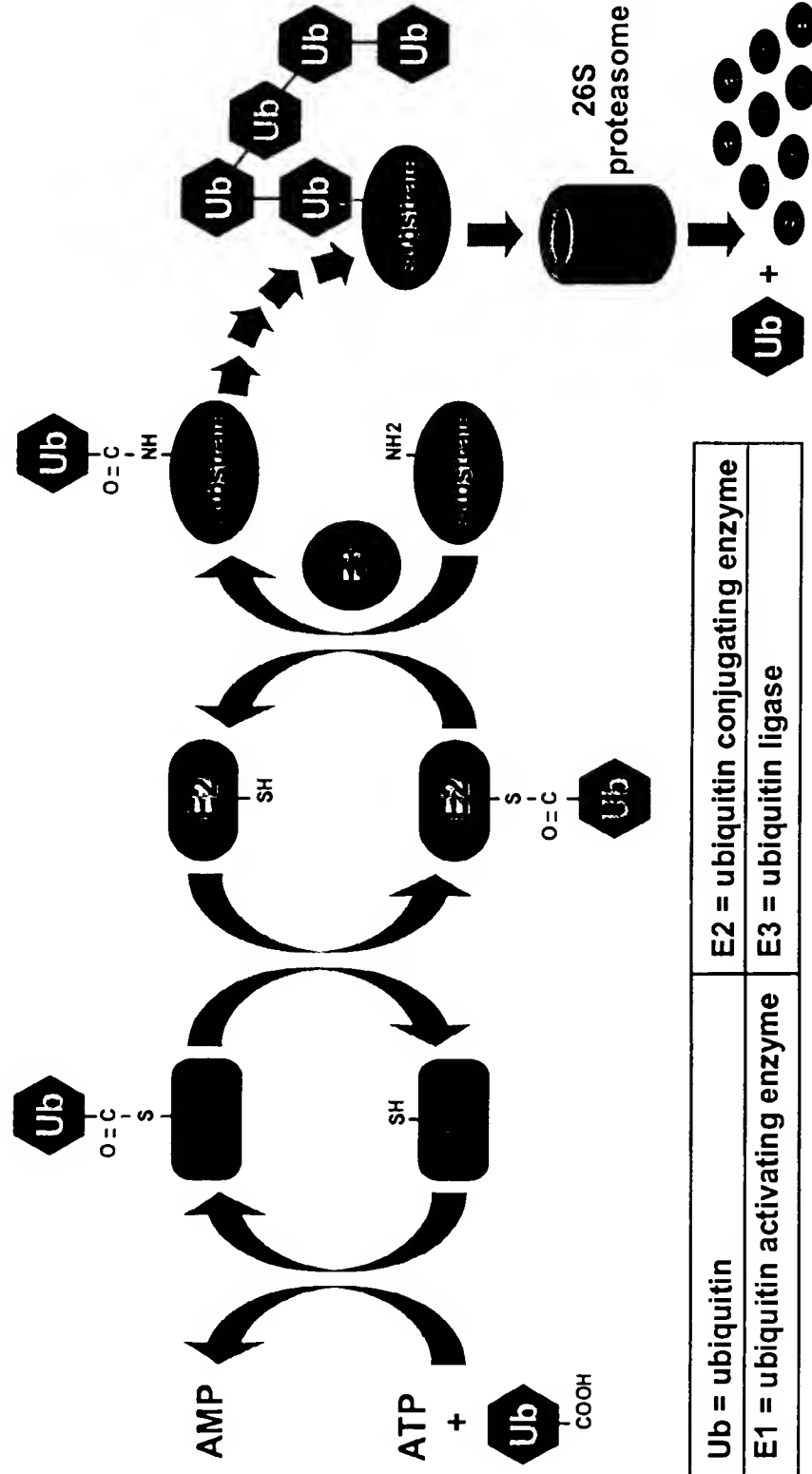
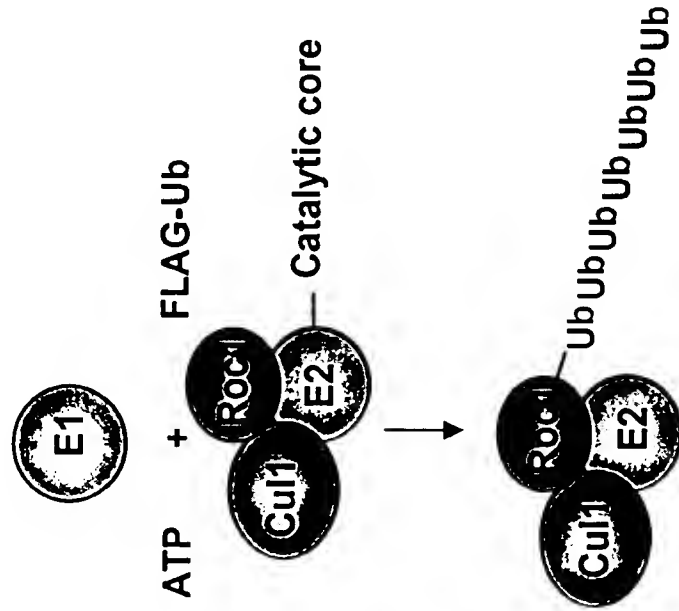


Figure 11A

A Reconstituted, Substrate-independent Assay for Studying Ligase Catalysis



The substrate-independent reaction has the same catalytic properties and requirements for Roc1/Cul1 as the substrate-dependent reaction

Reaction Components

E1:

E2 (UbcH5): GST-fusion (cleaved), E.coli

E3 (Ring/cullin): His-tagged, coexpressed, baculovirus

Ubiquitin: FLAG-tagged, E.coli

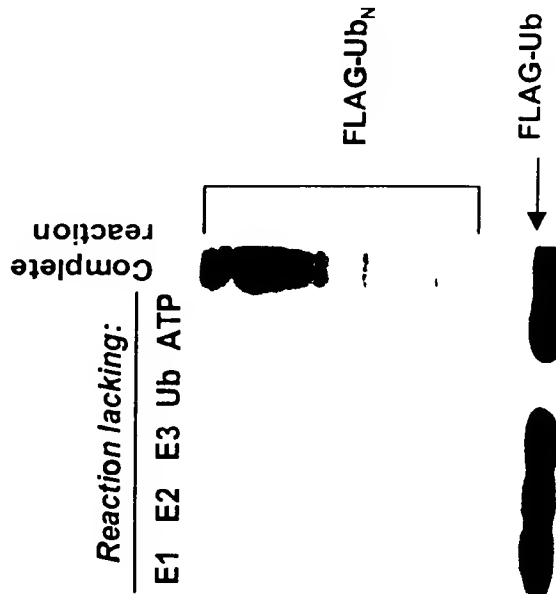
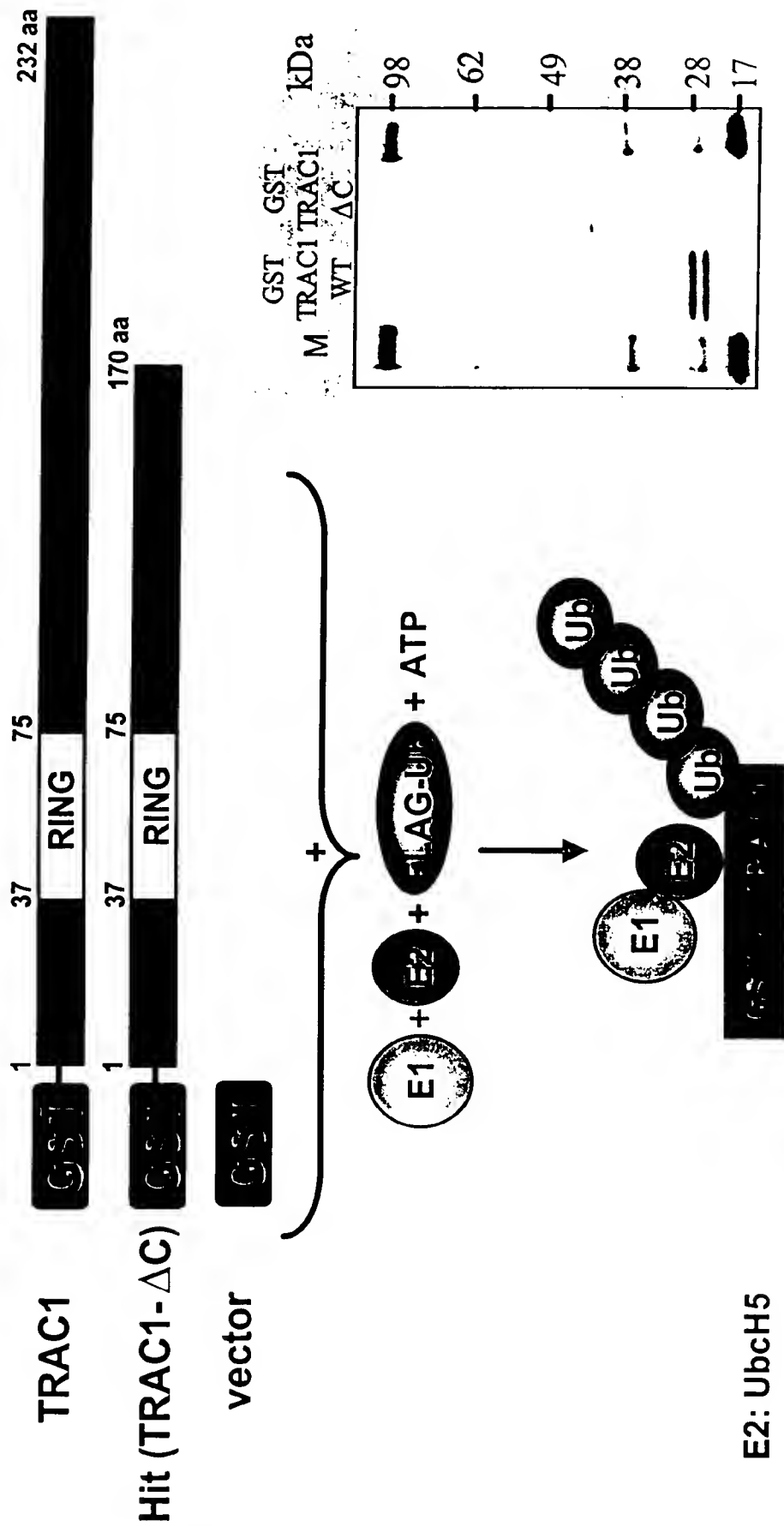


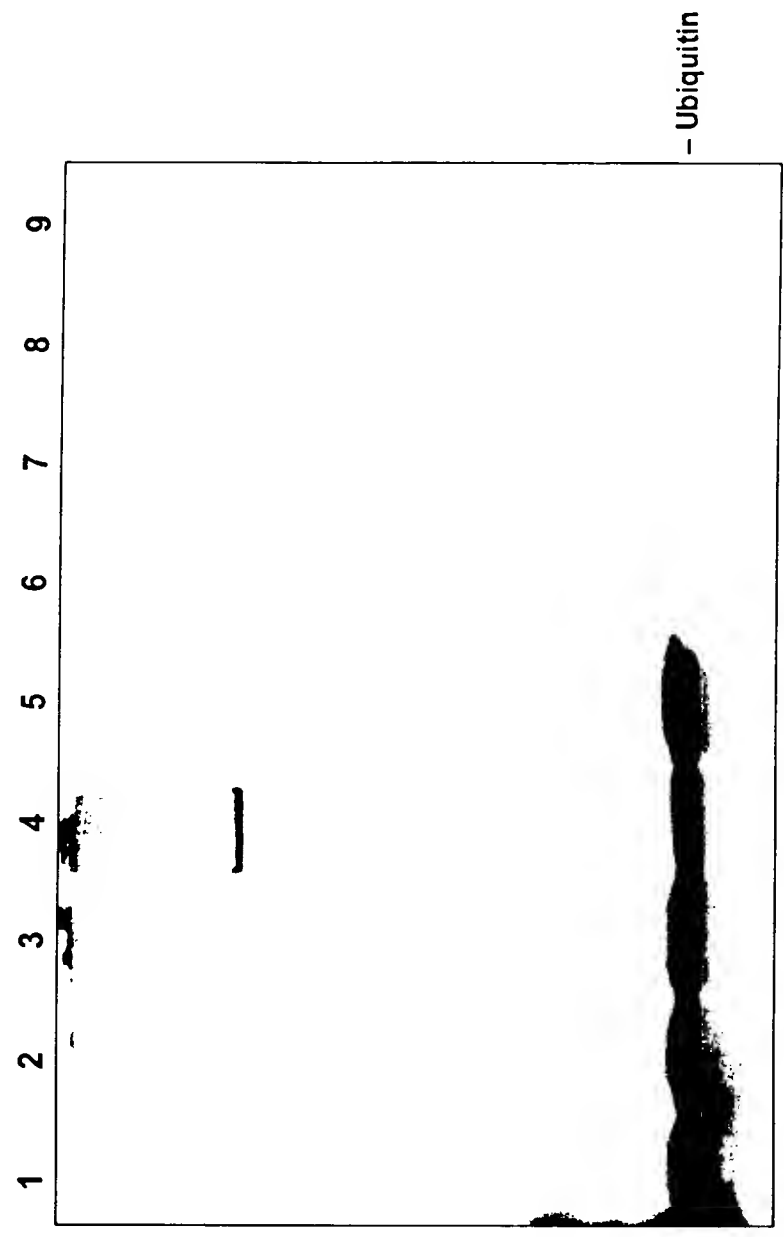
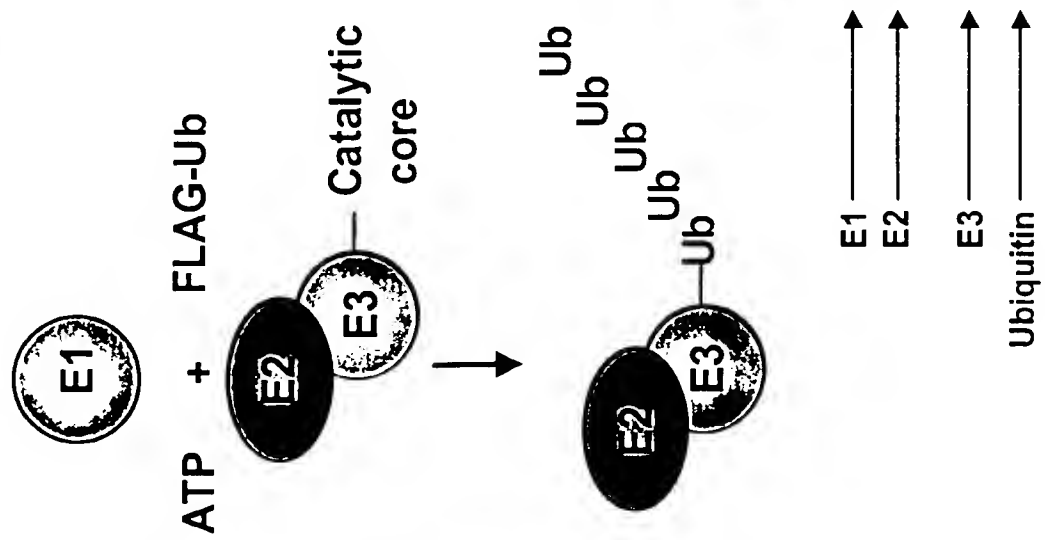
Figure 11B

Bacterially-expressed TRAC1 for Ligase Activity Assay



TRAC1 Exhibits E3 Ubiquitin Ligase Activity

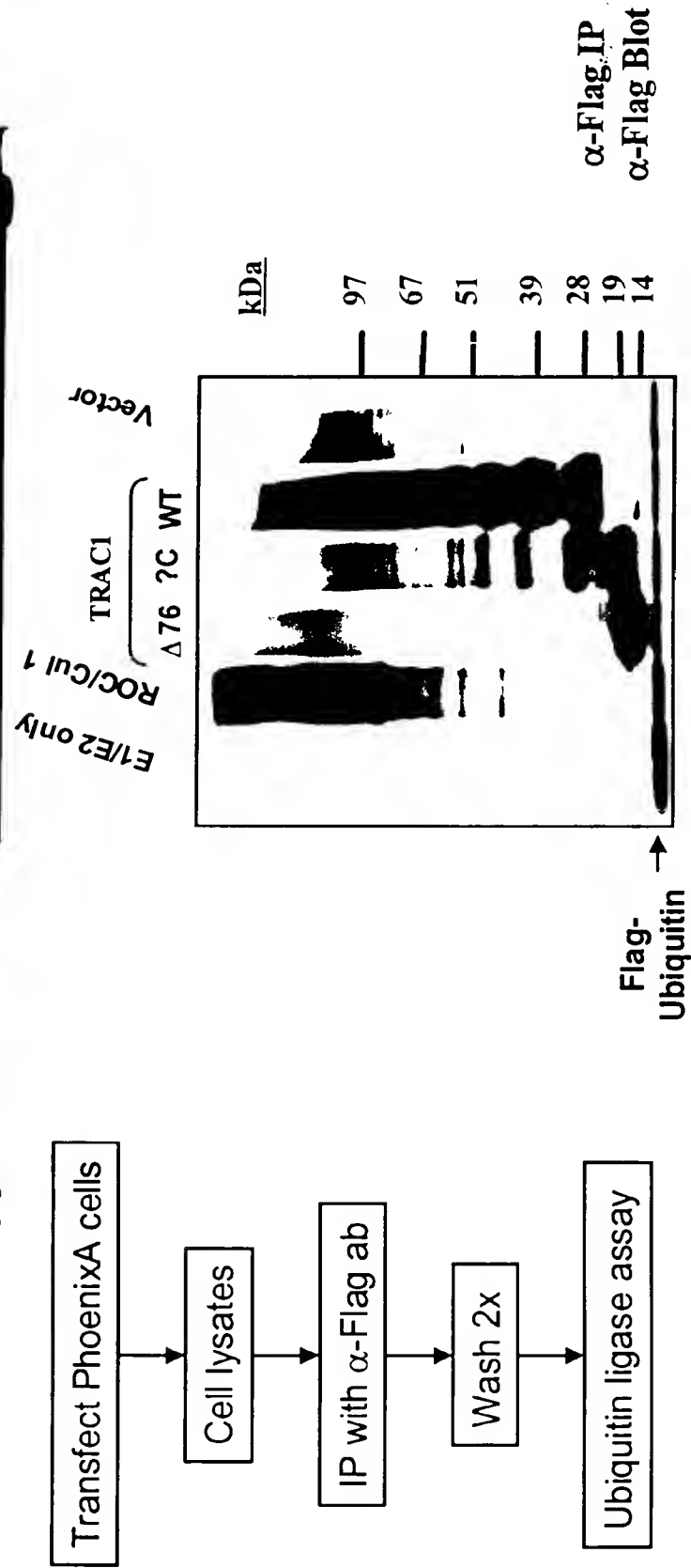
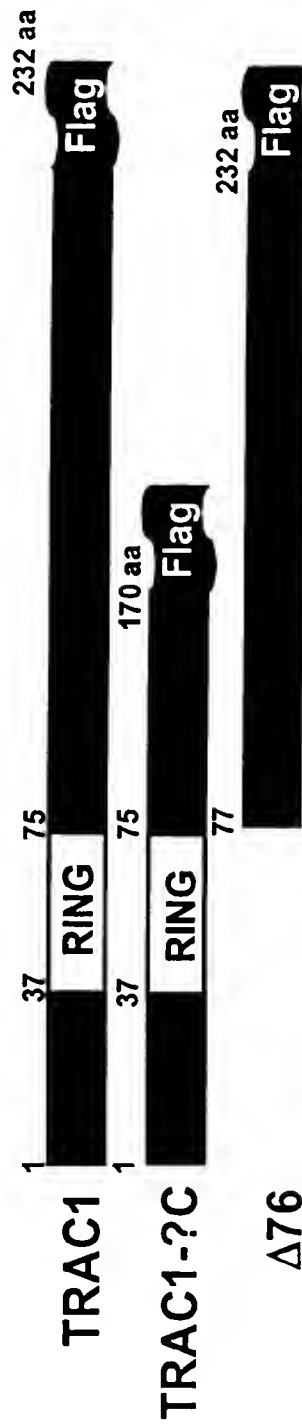
Figure 12A



+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+
	TRAC1 Δ	TRAC1 wt	ROC/Cul1	-	GST Vector	TRAC1 Δ	TRAC1 wt	ROC/Cul1
+	+	+	+	+	-	-	-	-

Figure 12B

The Ring Domain is Required for TRAC1 Ligase Activity



+

Point mutations in Conserved Cysteine Residues of the TRAC1 Ring finger Domain

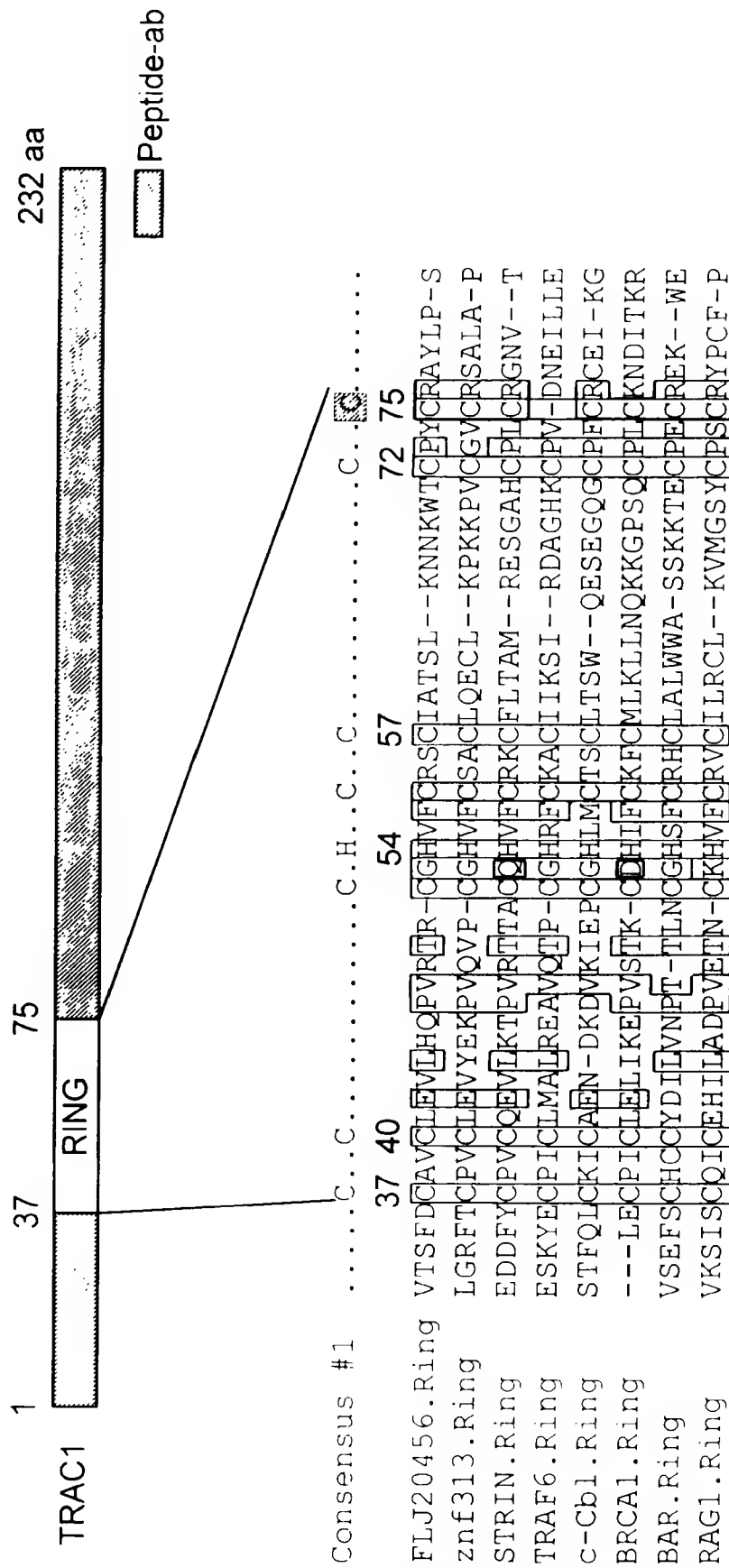


Figure 13B

Point mutations in Conserved Residues of the TRAC1 Ring-finger Domain Disrupt Ligase Activity

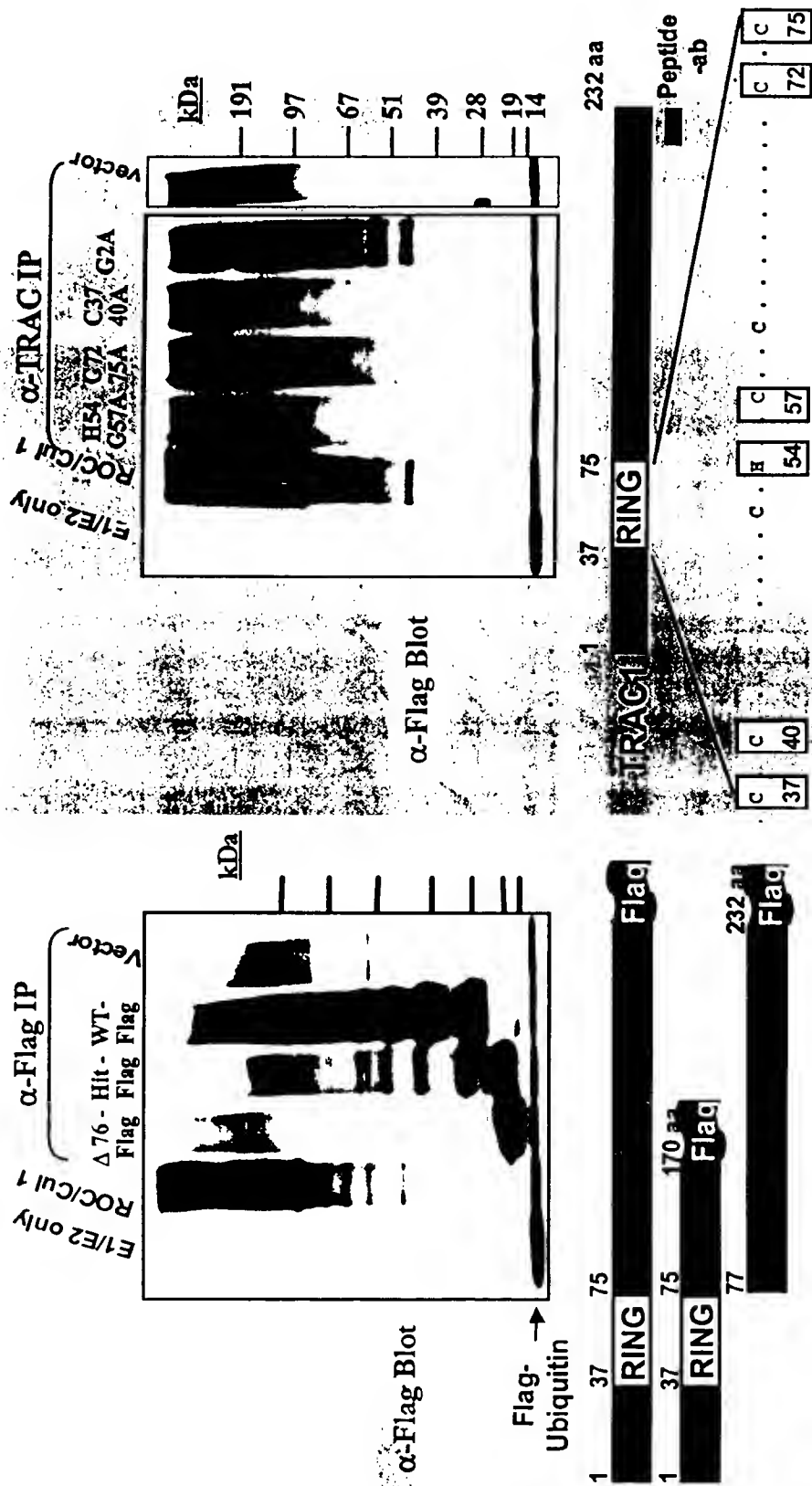


Figure 14

Expression of TRAC-1 mRNA is ~8 fold
higher in PBMC than in Jurkat cells

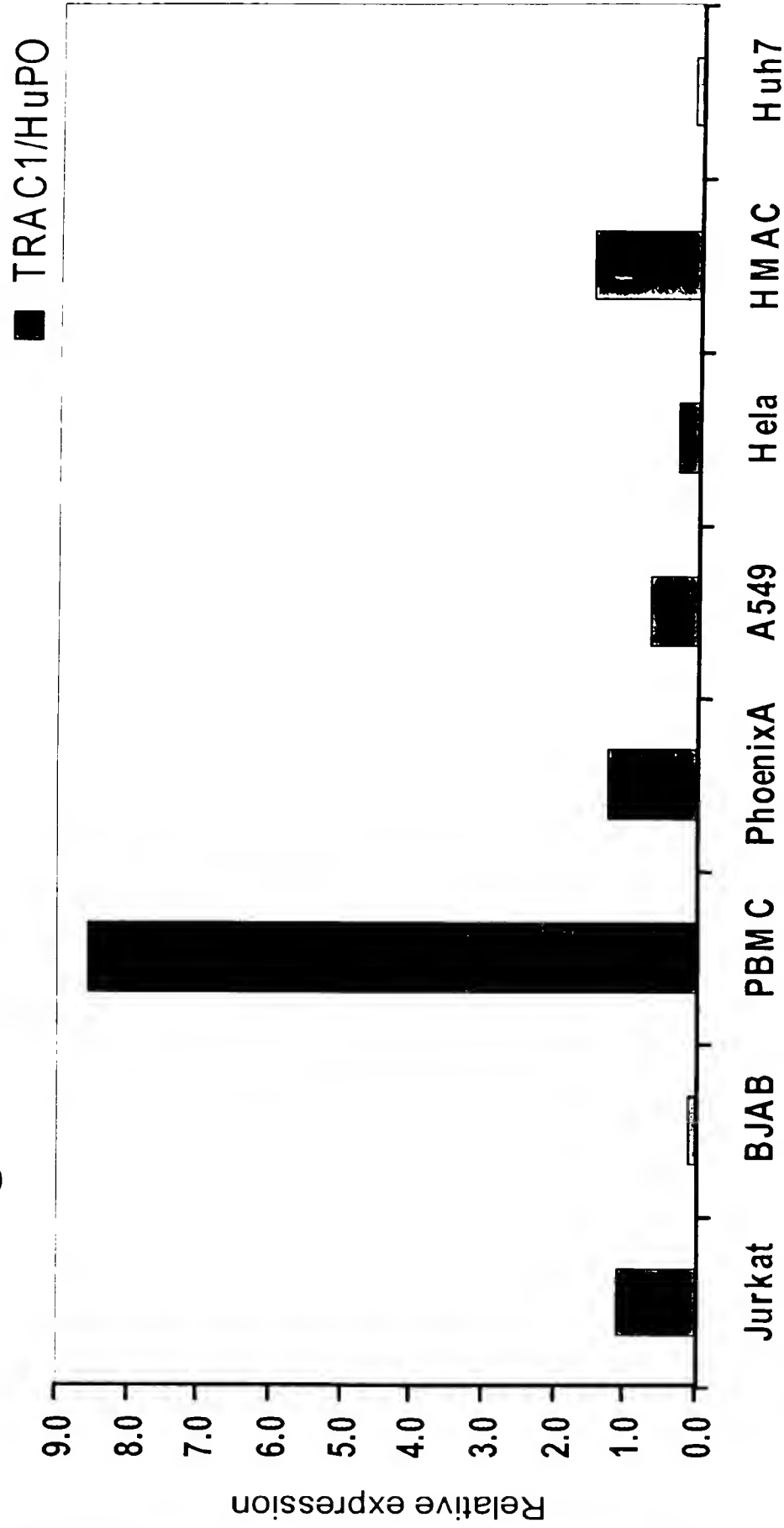


Figure 15A C-terminal Truncated TRAC1 Blocks TCR-induced Ca^{2+} Influx

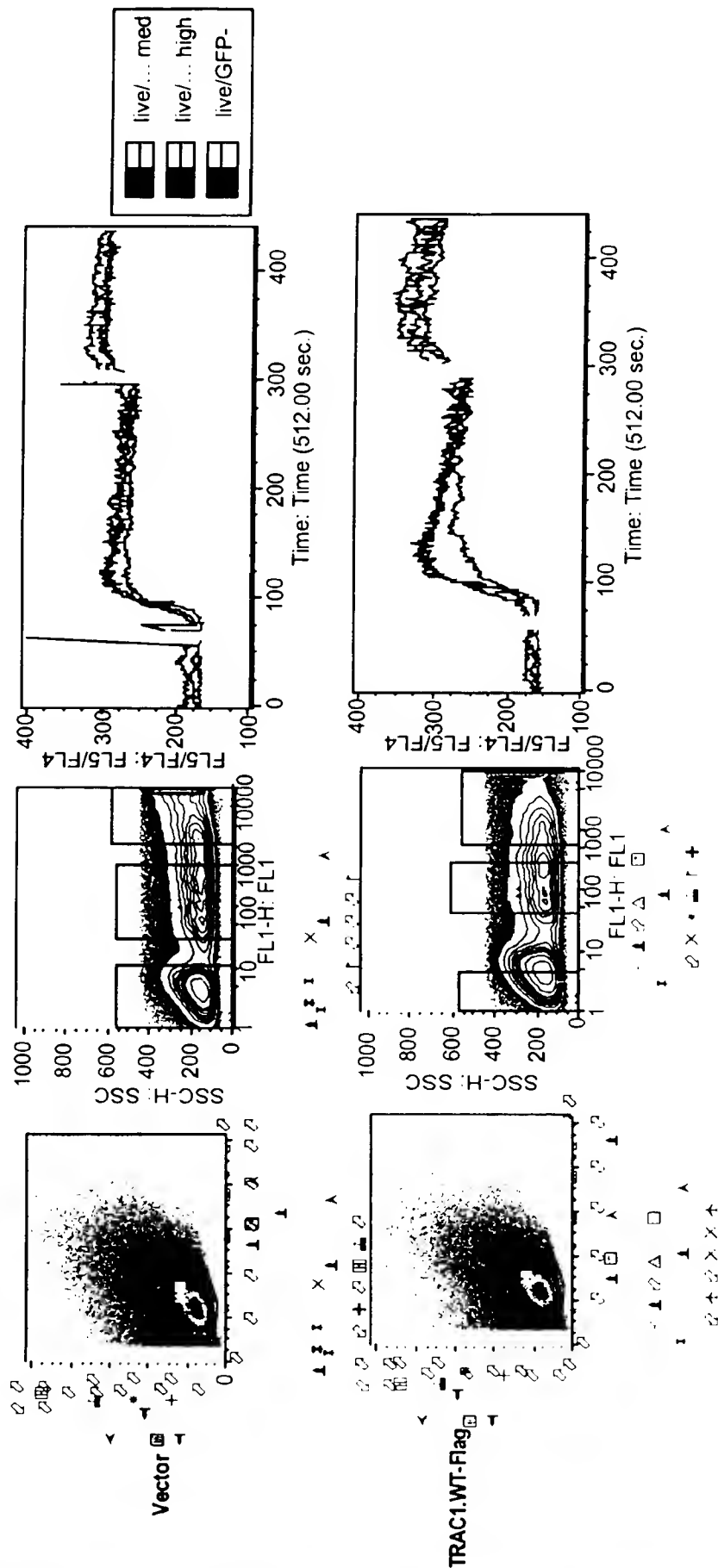


Figure 15B

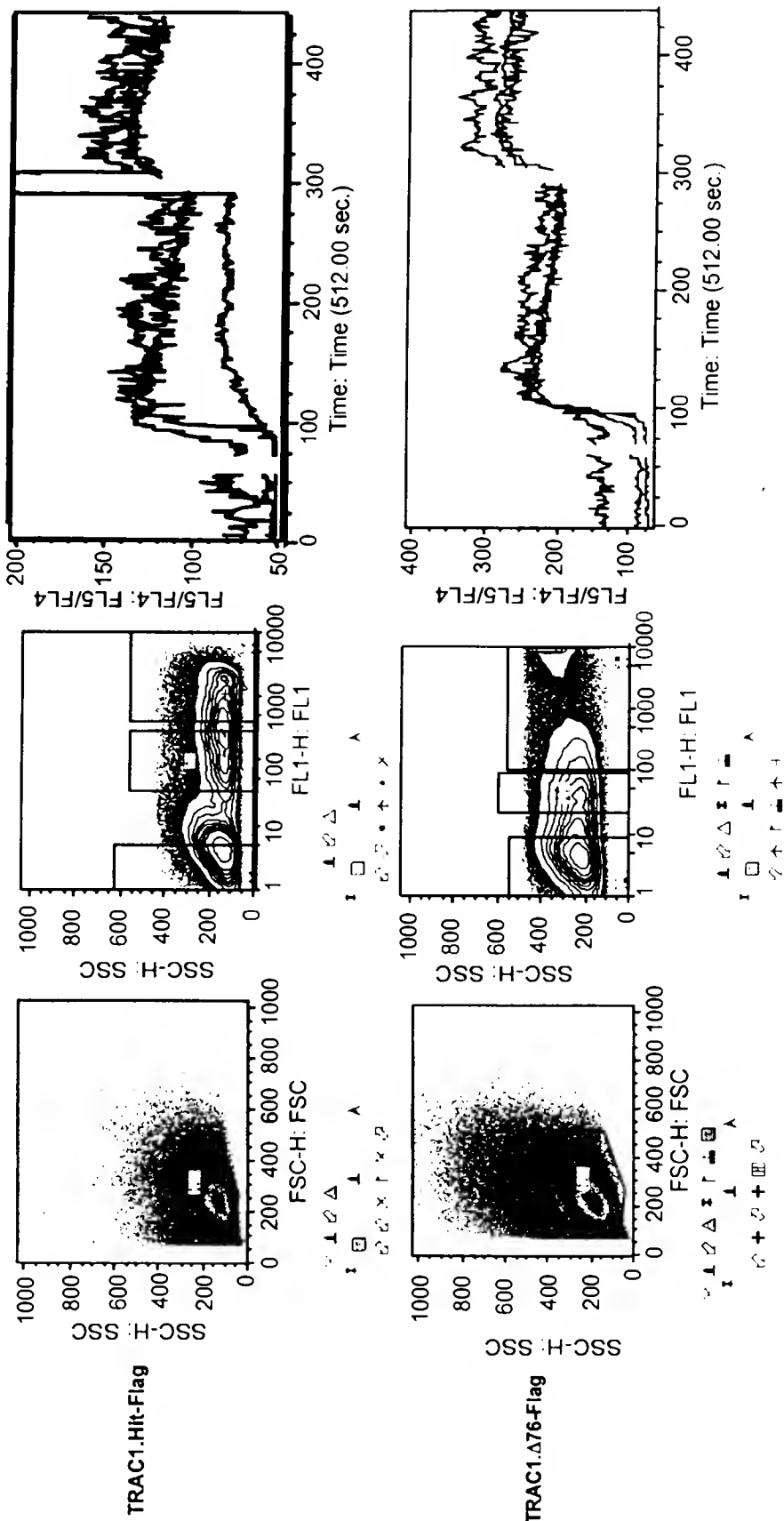


Figure 16

An Intact TRAC1 Ring domain is Required for Inhibition of α -TCR-Induced CD69 Up-regulation

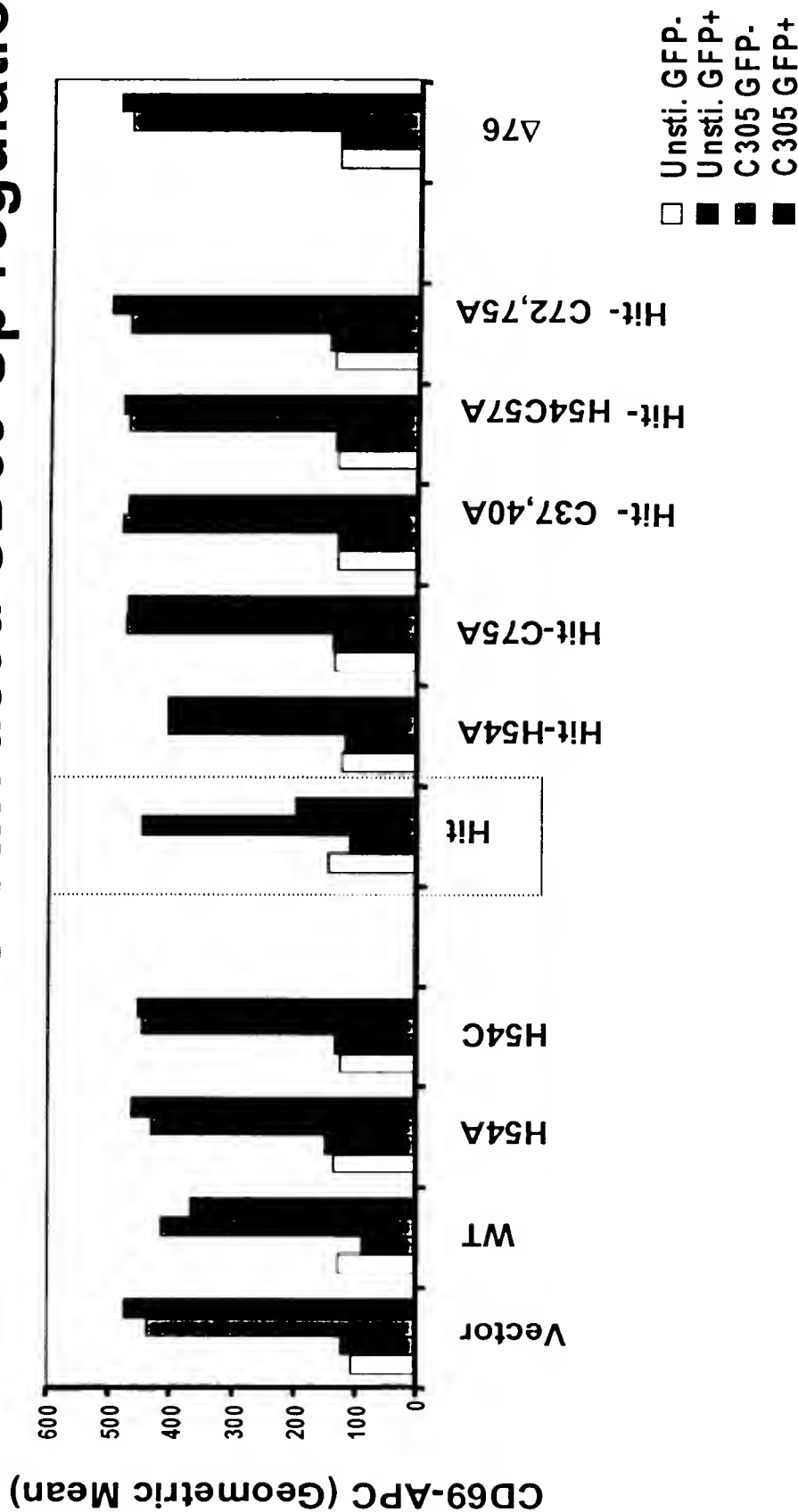


Figure 17

Summary of Functional Effects by Different TRAC-1 constructs

		Ubiquitin ligase activity	CD69 induction	Calcium mobilization
TRAC1	1 37 75 232 aa RING Flag	yes	-	+/-
Hit	1 37 75 170 aa RING Flag	yes	↓	↓
Δ76	77 232 aa Flag	no	-	-
C37,40A	XING Flag		-	-
C72,75A	RINX Flag		-	-
H54C57A	RXXG Flag		-	-

Figure 18

Transiently Transfected TRAC1 Protein Binds to Ubiquitin - Conjugating Enzymes (E2s) UbcH7 and UbcH5 *in vitro*

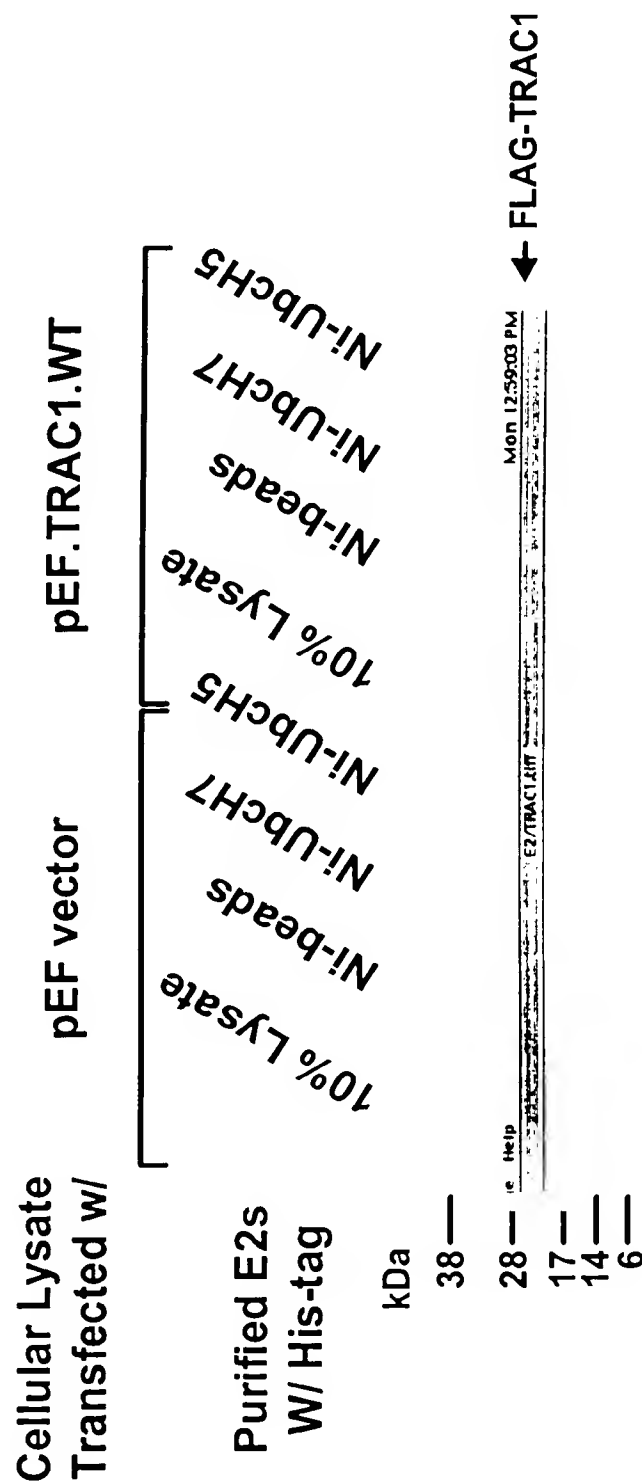


Figure 19

Model for TRAC-1 regulation of T cell activation

